

CLAIMS

1. An anti-proBNP(1-108) antibody, characterized in that, firstly, it specifically recognizes the sequence RAPR<sub>76</sub>S<sub>77</sub>P (SEQ ID No. 5) of proBNP(1-108) and does not substantially recognize the peptides BNP(1-76) or BNP(77-108) and, secondly, it has the ability to specifically recognize circulating proBNP(1-108) in human serum or plasma samples.
2. The anti-proBNP(1-108) antibody as claimed in claim 1, which specifically recognizes the sequence Y<sub>70</sub>TLRAPR<sub>76</sub>S<sub>77</sub>PKMVQGS<sub>85</sub> (SEQ ID No. 4) of proBNP(1-108).
3. The anti-proBNP(1-108) antibody as claimed in claim 1, which specifically recognizes the sequence Y<sub>70</sub>TLRAPR<sub>76</sub>S<sub>77</sub>PKMVQGS<sub>84</sub> (SEQ ID No. 108) of proBNP(1-108).
4. A method for obtaining an anti-proBNP(1-108) antibody as defined in one of claims 1, 2 and 3, in which an animal is immunized with the whole proBNP(1-108) molecule, and then the antiserum obtained is depleted using the BNP(77-108) peptide and/or the BNP(1-76) peptide.
5. A method for obtaining an anti-proBNP(1-108) antibody as defined in one of claims 1, 2 and 3, in which an animal is immunized with a peptide chosen from - a peptide of formula
- $$a_1-X_1-RAPRSP-X_2-a_2 \quad (I)$$
- where
- a<sub>1</sub> may be H or may represent a function or a chemical group chosen from a thiol, alcohol, aminoxy, primary amine or secondary amine function, an aminocarboxyl group, a biotinyl group and an acetyl group,
- X<sub>1</sub> represents a peptide sequence of 0 to 3 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

X<sub>2</sub> represents a peptide sequence of 0 to 7 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

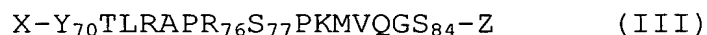
a<sub>2</sub> may represent an OH function, an NH<sub>2</sub> function or an alkoxy group;

- a peptide of formula



where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

- a peptide of formula



where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

- a peptide comprising a sequence derived from the sequence



X-Y<sub>70</sub>TLRAPR<sub>76</sub>S<sub>77</sub>PKMVQGS<sub>84</sub>-Z (III) by substitution of one or more among the amino acids Y<sub>70</sub>, T<sub>71</sub>, L<sub>72</sub>, K<sub>79</sub>, M<sub>80</sub>, V<sub>81</sub>, Q<sub>82</sub>, G<sub>83</sub>, S<sub>84</sub> and G<sub>85</sub>, with it being possible for X to be or to represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may be an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

- the peptide having the sequence C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S-G (C13P30: SEQ ID No.16);

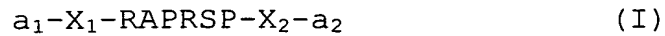
- the peptide having the sequence C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S (CN32: SEQ ID No. 109);

and, optionally, the antiserum obtained is depleted using the BNP(77-108) peptide and/or the BNP(1-76) peptide.

6. A method for obtaining a hybridoma that secretes an anti-proBNP(1-108) antibody as defined in one of

claims 1, 2 and 3, in which an animal is immunized with a peptide chosen from

- a peptide of formula



5 where

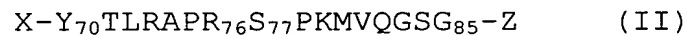
$a_1$  may be H or may represent a function or a chemical group chosen from a thiol, alcohol, aminoxy, primary amine or secondary amine function, an aminocarboxyl group, a biotinyl group and an acetyl group,

10  $X_1$  represents a peptide sequence of 0 to 3 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

$X_2$  represents a peptide sequence of 0 to 7 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

15  $a_2$  may represent an OH function, an  $NH_2$  function or an alkoxyl group;

- a peptide of formula



20 where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

25 - a peptide of formula



where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

30 - a peptide comprising a sequence derived from the sequence

$X-Y_{70}TLRAPR_{76}S_{77}PKMVQSG_{85}-Z$  (II) or from the sequence

35  $X-Y_{70}TLRAPR_{76}S_{77}PKMVQGS_{84}-Z$  (III) by substitution of one or more among the amino acids  $Y_{70}$ ,  $T_{71}$ ,  $L_{72}$ ,  $K_{79}$ ,  $M_{80}$ ,  $V_{81}$ ,  $Q_{82}$ ,  $G_{83}$ ,  $S_{84}$  and  $G_{85}$ , with it being possible for X to be or to represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108),

and where Z may be an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);  
- the peptide having the sequence C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S-G (C13P30: SEQ ID No.16);  
5 - the peptide having the sequence C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S (CN32: SEQ ID No. 109);  
immunoglobulin-secreting lymphocytes are taken from this animal,  
and the lymphocytes are fused with myeloma cells so as  
10 to obtain at least one immunoglobulin-secreting hybridoma.

7. The method as claimed in either of claims 5 and 6, in which the peptide of formula (II) has the sequence  
15 Y<sub>70</sub>TLRAPR<sub>76</sub>S<sub>77</sub>PKMVQGS<sub>85</sub> (SEQ ID No. 4).

8. The method as claimed in either of claims 5 and 6, in which the peptide of formula (III) has the sequence  
20 Y<sub>70</sub>TLRAPR<sub>76</sub>S<sub>77</sub>PKMVQGS<sub>84</sub> (SEQ ID No. 108).

9. A hybridoma which can be produced by the method as claimed in one of claims 6, 7 and 8.

10. An anti-proBNP(1-108) monoclonal antibody secreted  
25 by a hybridoma as claimed in claim 9.

11. A method of *in vitro* diagnosis of heart failure in a human, comprising bringing a biological sample into contact with an anti-proBNP(1-108) antibody as defined  
30 in one of claims 1, 2, 3 and 10, and detecting the proBNP(1-108) in the sample.

12. A method of *in vitro* diagnosis of heart failure in a human, comprising:

- 35 a) bringing a biological sample into contact with an anti-proBNP(1-108) antibody as defined in one of claims 1, 2, 3 and 10,  
b) incubating the mixture under conditions that allow the formation of antigen-antibody complexes, and

c) revealing the antigen-antibody complexes formed, optionally using a labeled detection antibody capable of binding specifically to the proBNP(1-108) present in the primary complex, or using a labeled detection antigen capable of binding to the antibody directed against said proBNP(1-108) present in the primary complex.

13. The method of diagnosis as claimed in claim 12, which also comprises a step d) for correlating the amount of antigen-antibody complexes revealed with the clinical condition of the individual.

14. A kit for detecting proBNP(1-108) in a biological sample, containing at least one antibody as defined in one of claims 1, 2, 3 and 10.

15. The kit for detecting proBNP(1-108) in a biological sample, as claimed in claim 14, containing:

(i) in a container, at least one antibody as defined in any one of claims 1, 2, 3 and 10;  
(ii) in another container, at least one peptide chosen from

- a peptide of formula  
25  $a_1-X_1-RAPRSP-X_2-a_2$  (I)  
where

$a_1$  may be H or may represent a function or a chemical group chosen from a thiol, alcohol, aminoxy, primary amine or secondary amine function, an aminocarboxyl group, a biotinyl group and an acetyl group,

30  $X_1$  represents a peptide sequence of 0 to 3 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

$X_2$  represents a peptide sequence of 0 to 7 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

35  $a_2$  may represent an OH function, an  $NH_2$  function or an alkoxyl group;

- a peptide of formula



where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an  
5 OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

- a peptide of formula



where X may be H or may represent either an acetyl  
10 group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

- a peptide comprising a sequence derived from the  
15 sequence

X-Y<sub>70</sub>TLRAPR<sub>76</sub>S<sub>77</sub>PKMVQSG<sub>85</sub>-Z (II) or from the sequence  
X-Y<sub>70</sub>TLRAPR<sub>76</sub>S<sub>77</sub>PKMVQGS<sub>84</sub>-Z (III) by substitution of one  
or more among the amino acids Y<sub>70</sub>, T<sub>71</sub>, L<sub>72</sub>, K<sub>79</sub>, M<sub>80</sub>, V<sub>81</sub>,  
Q<sub>82</sub>, G<sub>83</sub>, S<sub>84</sub> and G<sub>85</sub>, with it being possible for X to be  
20 or to represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may be an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

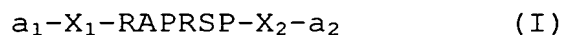
- the peptide having the sequence  
25 Y<sub>70</sub>TLRAPR<sub>76</sub>S<sub>77</sub>PKMVQSG<sub>85</sub> (SEQ ID No. 4);

- the peptide having the sequence  
Y<sub>70</sub>TLRAPR<sub>76</sub>S<sub>77</sub>PKMVQGS<sub>84</sub> (SEQ ID No. 109);

- the peptide having the sequence C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S-G (C13P30: SEQ ID No.16);

30 - the peptide having the sequence C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S (CN32: SEQ ID No. 108).

16. A peptide of formula:



35 where

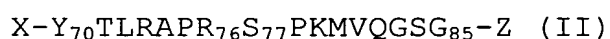
a<sub>1</sub> may be H or may represent a function or a chemical group chosen from a thiol, alcohol, aminoxy, primary amine or secondary amine function, an aminocarboxyl group, a biotinyl group or an acetyl group,

X<sub>1</sub> represents a peptide sequence of 0 to 3 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

5 X<sub>2</sub> represents a peptide sequence of 0 to 7 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

a<sub>2</sub> may represent an OH function, an NH<sub>2</sub> function, or an alkoxy group.

10 17. A peptide of formula



where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an  
15 OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108).

18. The peptide as claimed in claim 17, having the sequence Y<sub>70</sub>TLRAPR<sub>76</sub>S<sub>77</sub>PKMVQSGS<sub>85</sub> (SEQ ID No. 4).

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19. A peptide of formula:



where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the  
25 sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108).

20. The peptide as claimed in claim 19, having the  
30 sequence Y<sub>70</sub>TLRAPR<sub>76</sub>S<sub>77</sub>PKMVQGS<sub>84</sub> (SEQ ID No. 108).

21. A peptide comprising a sequence derived from the sequence X-Y<sub>70</sub>TLRAPR<sub>76</sub>S<sub>77</sub>PKMVQSGS<sub>85</sub>-Z (II) or from the sequence X-Y<sub>70</sub>TLRAPR<sub>76</sub>S<sub>77</sub>PKMVQGS<sub>84</sub>-Z (III) by substitution  
35 of one or more among the amino acids Y<sub>70</sub>, T<sub>71</sub>, L<sub>72</sub>, K<sub>79</sub>, M<sub>80</sub>, V<sub>81</sub>, Q<sub>82</sub>, G<sub>83</sub>, S<sub>84</sub> and G<sub>85</sub>, with it being possible for X to be or to represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may be an OH function, or 1

to 3 amino acids not belonging to the sequence of proBNP(1-108).

22. The peptide as claimed in claim 16, having a sequence chosen from the group consisting of the following sequences

SEQ ID No. 16: C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S-G  
(peptide C13P30)

SEQ ID No. 109: C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S  
(peptide CN32)

SEQ ID No. 6: C-G-R-A-P-R-S-P

SEQ ID No. 7: Acetyl-C-G-R-A-P-R-S-P

SEQ ID No. 8: C-G-R-A-P-R-S-P-K

SEQ ID No. 9: Acetyl-C-G-R-A-P-R-S-P-K

SEQ ID No. 10: C-G-R-A-P-R-S-P-K-M-V

SEQ ID No. 11: C-G-R-A-P-R-S-P-K-M-V-Q-G-S-G

SEQ ID No. 12: R-A-P-R-S-P-G-C

SEQ ID No. 13: Acetyl-R-A-P-R-S-P-G-C

SEQ ID No. 110: C-Y-T-L-R-A-P-R-S-P-K

SEQ ID No. 111: C-Y-T-L-R-A-P-R-S-P-K-M-V

SEQ ID No. 112: C-Y-T-L-R-A-P-R-S-P-K-M-V-Q

SEQ ID No. 113: C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G

SEQ ID No. 19: C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S- $\beta$ A

SEQ ID No. 20: C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-A-T- $\beta$ A

SEQ ID No. 114: Acetyl-C-T-L-R-A-P-R-S-P-K-M-V-Q

SEQ ID No. 115: C-T-L-R-A-P-R-S-P-K-M-V-Q-G

SEQ ID No. 116: C-T-L-R-A-P-R-S-P-K-M-V-Q-G-S

SEQ ID No. 117: C-T-L-R-A-P-R-S-P-K-M-V-Q-G-S-G

SEQ ID No. 118: C-L-R-A-P-R-S-P-K-M-V

SEQ ID No. 119: C-L-R-A-P-R-S-P-K-M-V-Q

SEQ ID No. 120: L-R-A-P-R-S-P-K-M-V-Q-C

SEQ ID No. 121: C-L-R-A-P-R-S-P-K-M-V-Q-G-S

SEQ ID No. 122: C-L-R-A-P-R-S-P-K-M-V-Q-G-S-G

23. A method for obtaining anti-proBNP(1-108) antibodies that specifically recognize the sequence Y<sub>70</sub>TLRAPR<sub>76</sub>S<sub>77</sub>PKMVQSG<sub>85</sub>, the sequence Y<sub>70</sub>TLRAPR<sub>76</sub>S<sub>77</sub>PKMVQGS<sub>84</sub> and/or the sequence RAPR<sub>76</sub>S<sub>77</sub>P of proBNP(1-108) with the substantial exclusion of the



BNP(1-76) and BNP(77-108) peptides, and that have the ability to specifically recognize circulating proBNP(1-108) in human serum or plasma samples, in which method an animal is immunized with a peptide as defined in one of claims 16 to 22,  
and, optionally, the antiserum obtained is depleted using the BNP(77-108) peptide and/or the BNP(1-76) peptide.

24. A method for obtaining a hybridoma that secretes an anti-proBNP(1-108) antibody that specifically recognizes the sequence  $Y_{70}TLRAPR_{76}S_{77}PKMVQGS_{85}$ , the sequence  $Y_{70}TLRAPR_{76}S_{77}PKMVQGS_{84}$  and/or the sequence  $RAPR_{76}S_{77}P$  of proBNP(1-108) with the substantial exclusion of the BNP(1-76) and BNP(77-108) peptides, and that have the ability to specifically recognize circulating proBNP(1-108) in human serum or plasma samples, in which method an animal is immunized with a peptide as defined in one of claims 16 to 22,  
immunoglobulin-secreting lymphocytes are removed from this animal,  
and the lymphocytes are fused with myeloma cells so as to obtain at least one immunoglobulin-secreting hybridoma.

25. An anti-proBNP(1-108) antibody, characterized in that it is obtained by a method as claimed in claim 23.

26. The anti-proBNP(1-108) antibody as claimed in claim 25, which specifically recognizes the sequence  $Y_{70}TLRAPR_{76}S_{77}PKMVQGS_{85}$  or the sequence  $Y_{70}TLRAPR_{76}S_{77}PKMVQGS_{84}$  of proBNP(1-108).

27. A hybridoma which can be produced by the method as claimed in claim 24.

28. An anti-proBNP(1-108) monoclonal antibody secreted by a hybridoma as claimed in claim 27.

29. The anti-proBNP(1-108) monoclonal antibody as claimed in claim 28, secreted by the hybridoma 3D4 deposited with the CNCM under the No. CNCM I-3073.

5 30. A method of *in vitro* diagnosis of heart failure in a human, comprising bringing a biological sample into contact with an anti-proBNP(1-108) antibody as defined in any one of claims 25, 26, 28 and 29, and detecting the proBNP(1-108) in the sample.

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31. A method of *in vitro* diagnosis of heart failure in a human, comprising:

a) bringing a biological sample into contact with an anti-proBNP(1-108) antibody as defined in one of claims  
15 25, 26, 28 and 29,

b) incubating the mixture under conditions that allow the formation of antigen-antibody complexes, and

c) revealing the antigen-antibody complexes formed, optionally using a labeled detection antibody capable  
20 of binding specifically to the proBNP(1-108) present in the primary complex, or using a labeled detection antigen capable of binding to the antibody directed against said proBNP(1-108) present in the primary complex.

25

32. The method of diagnosis as claimed in claim 31, which also comprises a step d) for correlating the amount of antigen-antibody complexes revealed with the clinical condition of the individual.

30

33. A kit for detecting proBNP(1-108) in a biological sample, containing at least one antibody as defined in one of claims 25, 26, 28 and 29.

35 34. A kit for detecting proBNP(1-108) in a biological sample, containing, as standard and/or control, at least one peptide as defined in one of claims 16 to 22.

35. A kit for detecting proBNP(1-108) in a biological sample, containing:

- in a container, at least one antibody as defined in one of claims 25, 26, 28 and 29;
- 5 - in another container, at least one peptide as defined in one of claims 16 to 22.